PLAGUE INFECTION IN A BRUSH-RAT (NEOTOMA FUSCIPES).*

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THE brush-rats are members of the same family, the Muridiae, to which the ordinary rats and mice belong; however, the genus Neotoma is classified under the subfamily, Neotominae, while the domestic rodents belonging to the genus Mus are assigned to the subfamily Murinae. In view of the well-known susceptibility to plague of members of the genus Mus, it is not surprising to find that this somewhat closely related rodent has been found infected, altho I am not acquainted with any previous report of this disease in any member of the genus Neotoma.

According to Elliot[1] Neotoma fuscipes (Baird) is found in the "Coast Region of California and Oregon, from Monterey Bay to the Columbia River." Other species of the same genus are found in various localities in the United States and in Mexico. Stephens[2] states that the type locality of Neotoma fuscipes (Baird) is Petaluma and Santa Clara, California, and that the species is "found from Monterery County north to Lake County" (California).

The following quotation relating to the habitat and to the habits of this rodent, which is generally known as the dusky-footed brush-rat, is taken from Stephens' *California Mammals:*

> Dusky-footed brush-rats inhabit the chemisal and the underbrush in open forests and groves, rarely being found in thick forests. This form does not appear to occur high in the mountains, seldom up to 3,000 feet altitude. The food is principally vegetable, but it is quite varied. They have the usual generic propensity for carrying off small articles.
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> The breeding season is March to June, perhaps later. The number of young in a litter is two to four. The home is usually in a "nest" or "house" of sticks, twigs, bones, or anything portable, these piles of rubbish being two to four feet high, roughly cone-shaped, and are usually placed in a thicket of brush, sometimes against a tree.
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> Occasionally the brush-rats take up their residence in barns or other buildings, where they do the most harm by carrying off small articles, stored vegetables, dried

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[1] *Synopsis of the Mammals of North America and the Adjacent Seas*, Field Columbian Museum, Chicago, 1901.

[2] *California Mammals*, The West Coast Publishing Company, San Diego, 1906.

fruits, grain, or anything they can carry off, even if utterly useless to them except to swell their rubbish pile. They seldom gnaw anything, however. They leave the premises immediately on the arrival of the introduced species of rat, which is a greater nuisance.

A small number (not over 40 or 50) of these animals have been examined at the Federal Laboratory at San Francisco, but only one presented any lesions that led to the suspicion of plague infection.

The rat in question was provisionally identified by my assistant, Mr. M. B. Mitzmain, as Neotoma fuscipes. For the confirmation of this provisional identification I am indebted to Professor Joseph Grinnell of the Department of Mammalogy of the University of California. The specimen was a full-grown male. Mention is made of the size, as a very considerable percentage of the grown domestic rats in this vicinity are immune to plague, and it may be a matter of interest at some future time to determine the susceptibility of large and small brush-rats.

Gross lesions.—The gross pathological changes were confined to the liver and to the right lung. The liver was literally crowded with fine yellowish-white granules, averaging somewhat smaller than a mustard-seed in size. The organ, aside from the granules, was rather ashen in color. The granules were similar to those often seen in plague in ordinary rats, but were larger and more prominent. They could not possibly have been overlooked by anyone familiar with the pathology of rodents, and in fact they at once attracted the attention of the laboratory assistant who was dissecting the animal.

The right lung was partially consolidated, dark red in color, and somewhat friable.

The spleen was apparently enlarged, but as we had no normal brush-rat for the purpose of comparison, no definite statement can be made on this point. It has been my experience that among presumably healthy rodents there is a great variation in the size of the spleen.

It is noteworthy that there was no bubo. A careful search was made for lesions of the lymph glands, but none was found.

The smears from the liver and from the spleen were negative; that is to say, they showed no organisms bearing any close resemblance to the pest bacillus. A smear from the consolidated lung tissue showed an enormous number of spherical bodies which agreed in size and in shape with the "coccoid" forms of B. pestis. A few fairly well-defined bipolar forms were also present. The organisms in general were grouped as if clumped, and not uniformly distributed over the field, as is usually the case in smears from plague tissue. All of these organisms were found to be decolorized when treated by Gram's method.

Cultures were not made from the organs of the animal as the smears showed that several organisms were present; therefore it was considered better to resort to the more certain method of animal

inoculation for the purpose of establishing a diagnosis. Two guinea-pigs were inoculated subcutaneously with tissues from the brush-rat. One guinea-pig was inoculated with lung tissue and died at the end of five days with lesions of acute plague. Cultures were contaminated. The second animal, inoculated with liver tissue, died at the end of five days with lesions of acute plague, and a pure culture of B. pestis was obtained from heart blood and liver.

It should be stated here that this experiment does not prove that plague bacilli were present in both of the tissues used for inoculation as the same instruments were employed throughout the dissection of the animal and the subsequent inoculations and it is possible that bacilli may have been present in one organ only (lung) and have been carried mechanically to the other.

The lesions in the guinea-pigs were entirely characteristic of plague. The organism isolated from the heart blood and from the liver gave a typical growth upon agar and in broth; upon 3 per cent salt agar the usual involution forms were developed in 24 hours. Litmus milk was unchanged even after two weeks' observation (difference from B. pseudotuberculosis rodentium [Pfeiffer]).

The result of the inoculation of the guinea-pigs made it desirable to carry out further experiments in order that the diagnosis might be placed upon as firm a foundation as possible. The next experiment was carried out with tissue that had been preserved in the ice-chest.

TABLE 1.

Animals	Mode of Inoculation	Day of Death	Lesions	Cultures
Guinea-pig.....	Cutaneous	7	Acute plague	Pure culture of B. pestis from heart blood and kidney
White rat.....	Cutaneous	5	" "	Pure culture of B. pestis from heart blood and spleen
White rat.....	Subcutaneous	Killed on 11th day	Chronic "	None made

The infection of a guinea-pig and of a white rat by the cutaneous method of inoculation furnished additional and very strong evidence in favor of the infecting organism being the plague bacillus; however, it seemed wise to determine the protective power of anti-pest serum against it.

RELATION TO ANTI-PEST SERUM.

No doubt was entertained now as to the nature of the organism, but as this was believed to be the first case of plague in a member of the genus Neotoma, we wished to make the evidence as convincing as possible. The full record of the work with the anti-pest serum is given, for altho it is realized that some of the results are of little value for the present purpose, they serve to illustrate some of the uncertainties in these procedures.

Series 1.—The specimen of anti-pest serum used in the first experiment was over two years old and the outcome of the test seems to indicate that it had but little protective power.

In each case the animal was vaccinated with the spleen of one of the white rats mentioned in the preceding experiment. This rat had died on the fifth day, and the spleen showed large numbers of pest-like bacilli. A pure culture of the organism was isolated from that organ. In this and in the subsequent experiments, the serum was given intraperitoneally immediately before the inoculation with the infecting organism. In each case the infecting organism was rubbed into the shaven belly.

TABLE 2.

Animal	Weight Grams	Serum Intraperitoneally	Day of Death	Lesions	Cultures
Guinea-pig.....	390	4 c.c. normal horse (control)	6	Acute plague	Pure culture of B. pestis isolated from kidney
" "	410	4 c.c. anti-pest	6	" "	Pure culture of B. pestis isolated from liver
White rat.....	150	4 c.c. normal horse	5	" "	Pure culture of B. pestis from heart blood
" "	145	4 c.c. anti-pest	Killed on 11th day	Chronic "	None made
" "	200	4 c.c. normal horse	5	Acute "	" "
" "	130	4 c.c. anti-pest	Killed on 11th day	None	" "
" "	105	4 c.c. normal horse	4	Acute plague	Contaminated
" "	130	4 c.c. anti-pest	5	Doubtful	"

In this case the serum had no influence upon the course of the disease in the "protected" guinea-pig. Two of the three "protected" rats survived; when killed one presented no lesions; the other had a purulent gland, which was interpreted as a lesion of chronic plague. The third "protected" rat died on the fifth day. The lesions were doubtful, and, unfortunately, the culture was contaminated. The

control rats all died of acute plague. Upon the whole, it may be said that this experiment no more than indicates that the serum exerted a considerable protective action upon the white rats, saving two out of three.

Series 2.—In the next experiment a comparatively fresh specimen of serum was used. The infecting material was a four-day-old broth culture isolated from the white rat (see Table 2) which had succumbed on the fifth day after cutaneous inoculation. The culture was the one derived from the same rat, the spleen of which furnished the infecting agent for the preceding experiment. The results are shown in the following table:

TABLE 3.

Animal	Weight Grams	Serum Intraperitoneally	Day of Death	Lesions
Guinea-pig.....	295	2 c.c. normal horse (control)	6	Acute plague
" "	270	2 c.c. anti-pest	12	Chronic "
" "	270	2 c.c. anti-pest	6	Acute "
White rat.....	70	2 c.c. normal horse (control)	Killed 13th day	Abscess at site
" "	80	2 c.c. anti-pest	Killed 13th day	No lesions

This experiment would lead one to conclude that anti-pest serum had practically no influence upon the course of the infection with the organism under consideration, altho one of the "protected" guinea-pigs lived twice as long as the control.

I am unable to say why the control white rat failed to succumb. There is no reason for believing that normal horse serum exerts any protective power against B. pestis.

Series 3.—A third and final series of inoculations was made, using a sample of anti-pest serum still more recent than that used in the preceding experiment. The results are shown in the next table. A

TABLE 4.

Animal	Weight Grams	Serum Intraperitoneally	Day of Death	Lesions
Guinea-pig.....	350	3 c.c. anti-pest	Killed on 35th day	No lesions
" "	433	" " "	11	Subacute plague
" "	380	Control, no serum	5	Acute "
" "	220	" " " "	4	" "
White rat*.....	...	3 c.c. anti-pest	Killed on 16th day	None
" "	...	" " "	" " " "	"
" "	...	" " "	" " " "	Small purulent focus in axillary gland
" "	...	Control, no serum	4	Acute plague
" "	...	" " "	6	" "
" "	...	" " "	6	" "

* The white rats were not weighed, but were all of approximately the same size.

broth suspension of a 48-hour agar culture isolated from one of the guinea-pigs in an earlier experiment was used as the infecting agent. The animals were all inoculated by the cutaneous method.

This experiment was entirely satisfactory in demonstrating the protective influence of the serum. One protected guinea-pig survived while the other markedly outlived the controls. The protected white rats survived, while the controls all died of acute plague.

GENERAL CONSIDERATIONS.

The question as to how the brush-rat was infected is one that cannot be answered now. Whether it was from another case of plague among its own species, or whether it was infected from a ground squirrel, or some other rodent, is purely a matter of conjecture. The rodent came from a county (Alameda) in which many infected squirrels have been found.

It would be idle to attempt to point out any special significance in the finding of plague in one of these rodents. It is perhaps worth while to call attention to the statement in the quotation from Stephens that "occasionally the brush-rats take up their residence in barns or other buildings." It is possible that by frequenting the habitations of man they may occasionally be the source of plague infection in human beings, either directly or through the ordinary house-rats.

The most that can be said at present is that the fact, now established, that these animals may have plague adds another factor to the already perplexing problem of the transmission of plague among the rodents of the Pacific Coast. Fortunately, the brush-rat is not sought for its flesh, as is the ground squirrel, nor does it ordinarily build its nests near human habitations as do the domestic rats.